PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	04.04.2006
Applicant's or agent's file reference PROBV/P32598PC	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2005/000751	28.02.2005	28.02.2004

Applicant
PROTHERICS MOLECULAR DESIGN LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PROBV/P32598PC	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/GB2005/000751	International filing date *(day/month/year)* 28.02.2005	Priority date *(day/month/year)* 28.02.2004

International Patent Classification (IPC) or national classification and IPC
INV. A61K31/517 A61K31/519 A61K38/48 A61P35/00

Applicant
PROTHERICS MOLECULAR DESIGN LIMITED et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in celectronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25.01.2006	04.04.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Ludwig, G Telephone No. +49 89 2399-8698

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2005/000751

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-38	as originally filed

Claims, Numbers

1-28	as originally filed
29-62	received on 19.01.2006 with letter of 18.01.2006

Drawings, Sheets

1/2, 2/2	as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-24, 53, 58

because:

☒ the said international application, or the said claims Nos. 1-24, 53, 58 relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-62
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-62
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-62 (cf. separate sheet)
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re item III.

1. Claims 1-24, 53, 58 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re item V.

2. In view of the state of the art as cited in the International search report the combination of antifolate compounds of formula I and of carboxypeptidase G (rescue agent) does not appear to be disclosed or suggested by the prior art.

Re Item VIII.

3. For the assessment of the present claims 1-24, 53, 58 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

29. Use according to any of Claims 25 to 28 for combating toxicity in an individual who has one of more clinical symptoms of toxicity caused by the antifolate compound.

30. Use according to Claim 29 wherein the clinical symptom of toxicity caused by the antifolate compound is selected from anaemia, anorexia, asthenia, dehydration, diarrhoea, fatigue, fever, hepatotoxicity, hyperbilirubinaemia, leukopaenia, mucositis, myelosuppression, nausea, neutropaenia, rash, reversible transaminitis, stomatitis, thrombocytopaenia and vomiting.

31. Use according to any of Claims 25 to 30 for combating toxicity in an individual who is administered a folate pathway rescue agent.

32. Use according to Claim 31 wherein the individual is administered the folate pathway rescue agent prior to the enzyme that has carboxypeptidase G activity.

33. Use according to Claim 31 wherein the individual is administered the folate pathway rescue agent after the enzyme that has carboxypeptidase G activity.

34. Use according to Claim 31 wherein the individual is administered the folate pathway rescue agent and the enzyme that has carboxypeptidase G activity substantially simultaneously.

35. Use of a folate pathway rescue agent in the preparation of a medicament for combating toxicity caused by an antifolate compound of Formula I as defined in Claim 1 or Claim 2 in an individual who is administered an enzyme that has carboxypeptidase G activity.

36. Use of an enzyme that has carboxypeptidase G activity and a folate pathway rescue agent in the preparation of a medicament for combating toxicity caused by an antifolate compound of Formula I as defined in Claim 1 or Claim 2.

37. Use according to any of Claims 31 to 36 wherein the antifolate compound is an inhibitor of DHFR or GARFT, and the folate pathway rescue agent is leucovorin.

38. Use according to Claim 37 wherein the antifolate compound is LY309887, AG2034, or AG2037.

39. Use according to any of Claims 31 to 36 wherein the antifolate compound of Formula I is an inhibitor of TS, and the folate pathway rescue agent is thymidine.

40. Use according to Claim 39 wherein the antifolate compound of Formula I is Tomudex.

41. Use according to any of Claims 25 to 40 wherein the enzyme that has carboxypeptidase G activity is at a dose of about 50 Units per kg body weight.

42. Use according to any of Claims 25 to 41 for combating toxicity caused by an antifolate compound of Formula I in an individual who is being treated for a disease selected from cancer, RA, MS, psoriasis, extrauterine pregnancy and graft vs. host disease by administration of the antifolate compound.

43. Use of an antifolate compound of Formula I as defined in Claim 1 or Claim 2 in the preparation of a medicament for treating a condition selected from cancer, RA, MS, psoriasis, extrauterine pregnancy and graft vs. host disease in an individual who is subsequently administered an enzyme that has carboxypeptidase G activity.

44. Use of an enzyme that has carboxypeptidase G activity in the preparation of a medicament for complementing the therapy of a disease selected from cancer, RA, MS, psoriasis, extrauterine pregnancy and graft vs. host disease that is being treated by administration of an antifolate compound of Formula I, wherein the medicament is for combating toxicity caused by the antifolate compound of Formula I.

45. Use according to any of Claims 42 to 44 wherein the antifolate compound of Formula I and the cancer to be treated are as defined in any of Claims 21-24.

46. A therapeutic system comprising an antifolate compound of Formula I as defined above in Claim 1 or 2, and an enzyme that has carboxypeptidase G activity.

47. A therapeutic system according to Claim 46 further comprising a folate pathway rescue agent.

48. An *ex vivo* method of cleaving a terminal L-glutamate moiety from a compound of Formula I as defined in Claim 1 or Claim 2, the method comprising contacting the compound with an enzyme that has carboxypeptidase G activity.

49. A method of determining the rate and/or extent of cleavage of a compound of Formula I as defined in Claim 1 or Claim 2 by an enzyme that has carboxypeptidase G activity, the method comprising:

providing the compound of Formula I,

contacting the compound of Formula I with an enzyme that has carboxypeptidase G activity under conditions such that cleavage of the compound can occur, and

monitoring the rate and/or extent of cleavage of the compound of Formula I over time.

50. A method according to Claim 49 wherein the monitoring step comprises monitoring the amount and/or concentration of the compound of Formula I.

51. A method according to Claim 49 or 50 wherein the monitoring step comprises monitoring the amount and/or concentration of one or more break-down products of the compound of Formula I.

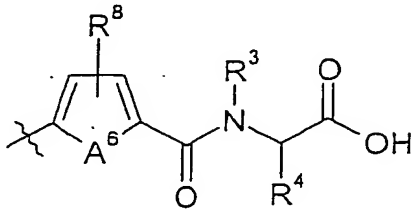
52. A method according to any of Claims 49 to 51 which is performed *ex vivo*.

53. A method according to any of Claims 49 to 51 which is performed *in vivo*.

54. A method according to Claim 53 further comprising determining whether an additional dose of the enzyme that has carboxypeptidase G activity is required in order reduce the amount of the compound of Formula I to a predetermined level.

55. A method according to Claim 53 or 54 further comprising contacting the compound of Formula I with an additional dose of the enzyme that has carboxypeptidase G activity under conditions such that cleavage of the compound can occur.

56. A method of cleaving a compound comprising a structural fragment of Formula VIII,



VIII

wherein

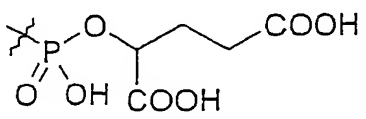
the wavy line indicates the point of attachment of the structural fragment;

A^6 represents O or S;

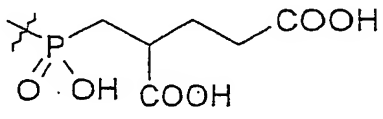
R^8 represents H or one or two substituents selected from halo, C_{1-4} alkyl and C_{1-4} alkoxy;

R^3 represents H or C_{1-4} alkyl;

R^4 represents $-CH_2C(R^{9a})(R^{9b})-D$;
R^{9a} and R^{9b} independently represent H or C_{1-4} alkyl, or R^{9a} and R^{9b} together represent $=C(H)R^{10}$;
R^{10} represents H or C_{1-4} alkyl;
D represents C(O)OH, tetrazol-5-yl, $(CH_2)_{0-1}$-NHR^{11}, or, when R^{9a} and R^{9b} together represent $=C(H)R^{10}$, then D may also represent H, or D represents a structural fragment of Formula IIIa or IIIb,



IIIa



IIIb ;

wherein the wavy lines indicate the point of attachment of the structural fragments;
R^{11} represents H or $C(O)R^{12}$;
R^{12} represents H or phenyl substituted by C(O)OH and optionally substituted by one or two further substituents selected from halo, C_{1-4} alkyl and C_{1-4} alkoxy; and

alkyl, alkenyl and alkynyl groups, as well as the alkyl part of alkoxy groups, may be substituted by one or more halo atoms;

or a pharmaceutically acceptable salt and/or solvate thereof,

the method comprising contacting the compound comprising the structural fragment of Formula VIII with an enzyme that has carboxypeptidase G activity.

57. A method according to Claim 56 that is performed *ex vivo*.

58. A method according to Claim 56 that is performed *in vivo*.

59. A method according to Claim 56 wherein the compound comprising the structural fragment of Formula VIII is an antifolate compound.

60. A method according to Claim 59 for combating toxicity caused by the antifolate compound in an individual who has been administered the said antifolate compound in the course of medical treatment, or otherwise, the method comprising administering to the individual an enzyme that has carboxypeptidase G activity.

61. Use of an enzyme that has carboxypeptidase G activity in the preparation of a medicament for combating toxicity caused by an antifolate compound of Formula VIII as defined in Claim 56.

62. A method according to any of Claims 1 to 24 or 48 to 60, or a use according to any of Claims 25 to 45 or 61, or a therapeutic system according to Claim 46 or 47, wherein the enzyme that has carboxypeptidase G activity is carboxypeptidase G_2, or a derivative thereof which has carboxypeptidase G activity.